Pergamon

Life Sciences, Vol. 56, No.s 11/12, pp. 853-859, 1995

0024-3205/95 $9.50 + .00

0024-3205(95)00020-8

TIOTROPIUM BROMIDE (Ba 679 BR), A NOVEL LONG-ACTING MUSCARINIC ANTAGONIST FOR THE TREATMENT OF OBSTRUCTIVE AIRWAYS DISEASE

Peter J Barnes, Maria G Belvisi, Judith CW Mak,
El-Bdaoui Haddad and Brian O'Connor

Department of Thoracic Medicine, National Heart and Lung Institute, London SW3 6LY, UK.

Summary

Tiotropium bromide (Ba 679 BR) is a novel potent and long-lasting muscarinic antagonist that has been developed for the treatment of chronic obstructive airways disease (COPD). Binding studies with [^{3}H]tiotropium bromide in human lung have confirmed that this is a potent muscarinic antagonist with equal affinity for M_1-, M_2- and M_3-receptors and is approximately 10-fold more potent than ipratropium bromide. Tiotropium bromide dissociates very slowly from lung muscarinic receptors compared with ipratropium bromide. *In vitro* tiotropium bromide has a potent inhibitory effect against cholinergic nerve-induced contraction of guinea-pig and human airways, that has a slower onset than atropine or ipratropium bromide. After washout, however, tiotropium bromide dissociates extremely slowly compared with the dissociation of atropine and ipratropium bromide. Measurement of acetylcholine (ACh) release from guinea-pig trachea shows that tiotropium bromide, ipratropium bromide and atropine all increase ACh release on neural stimulation and that this effect is washed out equally quickly for the three antagonists. This confirms binding studies to transfected human muscarinic receptors which suggested that tiotropium bromide dissociates slowly from M_3-receptors (on airway smooth muscle) but rapidly from M_2 autoreceptors (on cholinergic nerve terminals). Clinical studies with inhaled tiotropium bromide confirm that it is a potent and long-lasting bronchodilator in COPD and asthma. Furthermore, it protects against cholinergic bronchoconstriction for >24 h. This suggests that tiotropium bromide will be a useful bronchodilator, particularly in patients with COPD, and may be suitable for daily dosing. The selectivity for M_3- over M_2-receptors may also confer a clinical advantage.

Key Words: anticholinergics, muscarinic receptors, airways, chronic obstructive pulmonary disease, asthma

Cholinergic nerves are the dominant neural bronchoconstrictor pathways in human and animal airways (1). Acetylcholine (ACh) released from post-ganglionic nerve endings in the airways activates muscarinic receptors on target cells in the airways, including airway smooth muscle and submucosal glands (2). Cholinergic pathways may be activated reflexly via afferent nerves in the airways and outside the lungs and ACh release from nerve endings may be enhanced by several agents acting on pre-junctional receptors (3). Muscarinic receptor antagonists (loosely called anticholinergics) have been used for many years in the treatment of obstructive airways disease, including chronic obstructive airways disease (COPD) and asthma (4,5).

Current Use of Anticholinergics in Airway Disease

Anticholinergic drugs have been used for hundreds of years in the treatment of airway disease. Atropine and related compounds fell out of favor because of side effects (particularly central side effects such as hallucinations), but quaternary derivatives of atropine, such as ipratropium bromide and oxitropium bromide when given by inhalation are not absorbed from the respiratory tract or gastrointestinal tract and largely avoid the muscarinic side effects that limited the use of atropine (6). Muscarinic antagonists are used as maintenance bronchodilators in COPD, and to a lesser extent in asthma. In COPD the major reversible component is vagal cholinergic tone and anticholinergics are therefore as effective an ß-agonists. Indeed, several studies have demonstrated a superiority of anticholinergics over ß-agonists (4). In asthma, although there is an increase in airway cholinergic tone (7), there are other bronchoconstrictor mechanisms, including the direct constrictor effects of inflammatory mediators, such as histamine, cysteinyl-leukotrienes and thromboxane (8). For this reason $ß_2$-adrenergic agonists, that inhibit bronchoconstriction irrespective of the mechanism, are more effective bronchodilators than anticholinergics. However, in some situations anticholinergics are relatively more effective. In nocturnal exacerbations of asthma anticholinergics have some efficacy in a proportion of patients (9) and they may be more effective in the elderly. Infusion of atropine abolishes nocturnal bronchoconstriction in asthmatic patients, suggesting that vagal cholinergic tone is very important in contributing to the increased airway narrowing at night (10). Anticholinergics with a longer duration of action may therefore be more useful in controlling nocturnal asthma. Nebulized anticholinergics are almost as effective as $ß_2$-agonists in the emergency treatment of acute severe asthma, suggesting that cholinergic mechanisms become more important in acute exacerbations (11,12). Anticholinergics may also be more effective bronchodilators in infancy, as $ß_2$-receptors in the airways may not be fully developed.

Muscarinic Receptor Subtypes in Airways

Four muscarinic receptor subtypes (M_1-M_4) have been identified in the airways (13) and these receptor subtypes appear to play different functional roles (14). Autoradiographic mapping studies in animal and human lung have demonstrated that muscarinic receptors are localized predominantly to airway smooth muscle, submucosal glands, bronchial vessels and airway nerves (15-17). In human lung, in contrast to guinea-pig and ferret, there is also uniform labelling of the alveolar walls. Radioligand binding studies using selective antagonists have indicated that several subtypes of muscarinic receptor are present in human lung, with a predominance of M_1- and M_3-receptors (17,18). Autoradiographic mapping shows that muscarinic receptors are differentially localized in guinea-pig and human lung. M_1-receptors are localized to submucosal glands, parasympathetic ganglia and, in human lung, to alveolar walls, whereas M_3-receptors are localized to airway smooth muscle, bronchial vascular endothelium, submucosal glands and, to a lesser extent, airway epithelial cells (17). M_2-receptors have limited expression in human lung, with scanty labelling of airway smooth muscle, although this is more marked in guinea-pig airway smooth muscle, especially in peripheral airways. This binding data has been supported by studies using molecular (cDNA) probes for muscarinic receptor subtypes. *In situ* hybridization has revealed that m1 mRNA is expressed predominantly in alveolar walls in human airways, whereas m3 mRNA is expressed in airway smooth muscle and submucosal glands (19). m2-receptor mRNA is expressed predominantly in airway smooth muscle, particularly in more peripheral airways, whereas m4- and m5-receptor mRNA are not expressed in human lung (19). There appear to be marked differences between species. In rabbit there is strong expression of m4-receptor mRNA in the lung (20) and this is consistent with binding studies in rabbit lung (21). *In situ* hybridization demonstrates expression of m4-receptor mRNA in pulmonary vascular and airway smooth muscle (20). In porcine lung M_2-receptors and m2-receptor mRNA predominate in lung parenchyma, with m2- and m3-receptor mRNA in airway smooth muscle (22).

Muscarinic receptor subtypes in the airways appear to be involved in the regulation of different functions. M_1-receptors appear to be facilitatory to ganglionic transmission and enhance cholinergic reflex effects in human airways (23). Although the m1-receptor antagonist pirenzepine has been reported to have a bronchodilator effect in asthmatic patients after intravenous administration (24), the longer-acting drug telenzepine had no significant bronchodilator action in COPD (25). M_1-receptors are also likely to be involved in mucus gland secretion, although M_1-receptors are less numerous than M_3-receptors (17). In human lung 70% of receptors in peripheral lung are of the M_1-receptor subtype (26) and M_1-receptors are also present in porcine lung parenchyma (22). It seems unlikely that these peripheral receptors have any physiological role as there is no cholinergic innervation of lung parenchyma.

The bronchoconstrictor response to cholinergic agonists is mediated via M_3-receptors that stimulate phosphoinositide hydrolysis (27), and this is consistent with the expression of M_3-receptors on human airway smooth muscle (17,19). Mucus gland secretion is also mediated predominantly via M_3-receptors (28). Binding studies also indicate that there is a high proportion of M_2-receptors in airway smooth muscle, which are coupled via G_i to adenylyl cyclase, resulting in a decrease in cyclic AMP (29). This suggests that cholinergic agonists may oppose ß-adrenergic receptor-mediated bronchodilatation, and although this has been described in some species (30,31), it has not been observed in others (32). The role of M_2-receptors in human airway smooth muscle is not yet certain and the amount of m2-receptor expression in human airway smooth muscle is less pronounced than in other species (17,19).

Muscarinic autoreceptors that inhibit the release of ACh from post-ganglionic nerve endings have been described in the airways of several species (3,14). These autoreceptors have also been demonstrated functionally in human airways by contraction studies (33) and more directly by measurement of ACh release on electrical field stimulation (34). In guinea-pig airways the relative potency of cholinergic antagonists in blocking autoreceptor function suggests that these receptors are of the M_2-, or possibly of the M_4-receptor subtype (34-36). In human airways the receptors appear to be M_2-receptors (34). Non-selective muscarinic antagonists may therefore increase the amount of ACh released by nerve stimulation and this may weaken their blockade of post-junctional M_3-receptors in airway smooth muscle. Thus, after ipratropium bromide there is an increase in ACh release in human airways (34). This is a relative disadvantage of such non-selective muscarinic antagonists. While this may not be important in the acute protective effect of these drugs against cholinergic reflex bronchoconstriction, it may become important as the effect of the drug wears off.

There is some evidence that muscarinic autoreceptors may become dysfunctional in experimental animals under certain conditions, including virus infections, allergen exposure and with eosinophil major basic protein (37). This would lead to increased cholinergic bronchoconstriction. There is also evidence that muscarinic autoreceptors may be dysfunctional in patients with asthma (38,39). If this is the case non-selective anticholinergic drugs would not increase ACh in patients with asthma, but may cause problems in patients with COPD where drugs are used most widely. This suggests that anticholinergic antagonists selective for M_3-receptors (and also M_1-receptors), that avoid blockade of M_2-receptors may have an advantage over non-selective drugs such as ipratropium bromide, in the treatment of patients with COPD.

Tiotropium bromide (Ba 679 BR)

Tiotropium bromide (Ba 679 BR, Boehringer Ingelheim), a long-acting muscarinic antagonist has recently been developed. Its quaternary ammonium structure is derived from that of ipratropium bromide (figure 1). In a series of pharmacological studies tiotropium was shown to be a potent muscarinic receptor antagonist, with a prolonged duration of blockade in guinea-pig trachea *in vitro* and after inhalation in dogs *in vivo* (40). In Chinese hamster ovary (CHO) cells transfected with human muscarinic receptor subtype cDNAs, apparent binding affinity (K_D) of tiotropium bromide and ipratropium bromide were similar for hm1, hm2 and

hm3 receptors, but kinetic studies (at 23°C) showed that [^{3}H]tiotropium bromide dissociated over 100 times more slowly that [^{3}H]ipratropium bromide from hm1 (14.6 *vs* 0.11 h) and hm3 (34.7 *vs* 0.26 h), whereas dissociation from hm2 (0.035 *vs* 3.6 h) was more similar (40). This suggests that tiotropium bromide has a kinetic selectivity for M_1- and M_3-receptors over M_2-receptors.

TIOTROPIUM BROMIDE (Ba 679) IPRATROPIUM BROMIDE

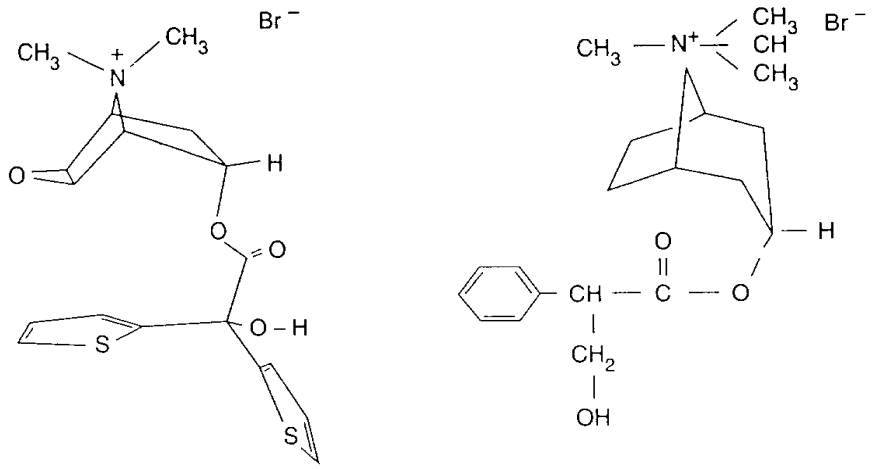

Figure 1. Structures of tiotropium bromide and ipratropium bromide

Binding Studies in Human Lung

We determined the affinity, selectivity, kinetics and distribution of tiotropium bromide binding in human lung (41). [^{3}H]tiotropium bromide bound with high affinity to a uniform population of muscarinic receptors in human peripheral lung membranes. The binding affinity of [^{3}H]N-methyl scopolamine (NMS) was approximately 6-fold lower, and competition studies showed that tiotropium bromide was approximately 10-fold more potent than ipratropium bromide and atropine in displacing specific [^{3}H]NMS binding. There was no evidence for selectivity in the binding of [^{3}H]tiotropium bromide to rat cerebrocortical M_1-receptors labelled with [^{3}H]telenzepine, or heart M_2- and salivary gland M_3-receptors, labelled with [^{3}H]NMS. Tiotropium bromide had a long-lasting protective effect against [^{3}H]NMS binding and this lasted for >90 min, whereas ipratropium bromide had little protective effect. Similarly, [^{3}H]tiotropium bromide dissociated extremely slowly from human lung membranes, with a half life (at 30°C) of almost 4 h.

Autoradiographic mapping of [^{3}H]tiotropium bromide in human lung sections, showed labelling of alveolar walls and submucosal glands, with little specific labelling of airway smooth muscle or epithelium. Both pirenzepine and 4-DAMP displaced specific binding, but methoctramine was without any effect, suggesting that M_1- and M_3-receptors were labelled.

Effects on Cholinergic Neurotransmission in Vitro

We have also investigated the effect of tiotropium bromide on cholinergic neural responses in guinea-pig and human airways *in vitro* (42). Tiotropium bromide potently inhibited cholinergic nerve-induced contraction of guinea-pig trachea and was approximately 5-fold more potent than ipratropium bromide or atropine. The onset of action of tiotropium bromide was slower than seen with atropine or ipratropium bromide and, after washout, its duration of action in blocking cholinergic neural responses was greatly prolonged, with a t½

of 540 min, compared with 81 min for ipratropium bromide. In human bronchi tiotropium bromide had a similar inhibitory effect and was 10 times more potent than atropine. Again, the onset of action was slow compared with atropine, and its offset very prolonged ($t^1/_2$ >300 min) compared to atropine ($t^1/_2$ 64 min) (figure 2). These studies indicate that tiotropium bromide has a very prolonged inhibitory effect against endogenous ACh released from post-ganglionic nerve endings in the airways, presumably via an inhibitory effect on post-junctional M_3-receptors.

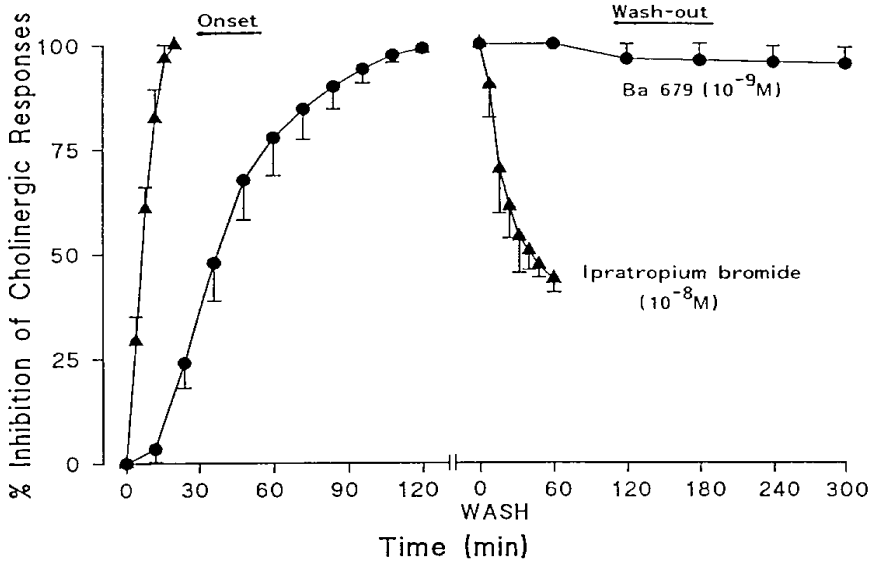

Figure 2. Onset and wash-out of inhibitory effects of tiotropium bromide (Ba 679, 10^{-9}M) and ipratropium bromide (10^{-8}M) on cholinergic neural responses in human bronchi *in vitro*. Mean values ± SEM of 5 separate subjects are shown.

We also studied the effect of tiotropium bromide on ACh release after electrical field stimulation in guinea-pig airways. Electrical field stimulation increased ACh release, measured by a [^{3}H]choline superfusion technique, by approximately 6-fold (43). Tiotropium bromide, ipratropium bromide and atropine all increased ACh release to a similar extent (30-40%), but this was lost two hours after wash-out of the antagonists. Thus, although tiotropium bromide causes prolonged blockage of airway smooth muscle M_3-receptors after wash-out, this not appear to apply pre-junctional muscarinic autoreceptors (M_2 or M_4). This demonstrates the kinetic selectivity of tiotropium bromide, first demonstrated in binding studies to transfected cells (40), also applies in *in vitro* functional studies.

Clinical Studies

The effect of inhaled tiotropium bromide has also been studied in clinical studies in patients with COPD and asthma. In 6 patients with COPD inhaled tiotropium bromide was found to cause a dose-related bronchodilator response which persisted for almost 24 h at the highest doses used (44). The drug was well tolerated and no significant side effects were reported at single doses of up to 160 μg; in normal volunteers no adverse effects have been reported in doses up to 200 μg (45). In a recent study of 12 patients with asthma, we have confirmed the prolonged bronchodilator effect of inhaled tiotropium bromide and have also demonstrated a prolonged dose-dependent protection against inhaled methacholine challenge (46). At an inhaled dose of 40μg there was a protection of over 7 doubling dilutions against methacholine and protection lasted for 48 h. This should be compared with a protective effect of oxitropium bromide of less than 6 h (47). There were no adverse effects of inhaled tiotropium bromide and no effects on heart rate or blood pressure.

These clinical studies support the animal and *in vitro* studies and show that tiotropium bromide is a potent and long-lasting antimuscarinic agent. It is likely to be a useful addition to the therapy of COPD, where once daily administration may prove to be more convenient than the currently recommended three to four times daily treatment needed for ipratropium bromide. The prolonged protection against cholinergic neural bronchoconstriction may also be useful in the control of nocturnal asthma, where cholinergic mechanisms appear to be important (10). Whether the kinetic selectivity for M_1- and M_3-receptors over M_2-receptors will be useful clinically remains to be determined. Side effects do not appear to be a problem at doses that are useful clinically, although it will be important to protect against eye contact, so that a dry powder inhaler formulation rather than a metered dose inhaler, may be the most appropriate. Further studies are now indicated looking at more prolonged dosing, and monitoring of effects on airway function and mucus secretion.

Acknowledgements

We thank Medical Research Council (U.K.) and Boehringer Ingelheim for support.

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